Scand J Clin Lab Invest 2000; 60: 367–380

Examination of four different instruments for measuring blood lactate concentration

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Medbø JI, Mamen A, Holt Olsen O, Evertsen F. Examination of four different instruments for measuring blood lactate concentration. Scand J Clin Lab Invest 2000; 60: 367–380.

Information on the performance of different instruments used to measure blood lactate concentration is incomplete. We therefore examined instruments from Yellow Springs Instruments (YSI 23L and YSI 1500) and three cheaper and simpler instruments: Dr. Lange's LP8+, Lactate Pro from Arkray in the KDK corporation and Accusport from Boehringer Mannheim. First, a number of blood samples were analysed by standard enzymatic photofluorometry (our reference method) and, in addition, by one or more of the instruments mentioned above. Second, measurements using two or more identical instruments were compared. Third, since Lactate Pro and Accusport are small (≈100 g, pocket-size), battery-driven, instruments that could be used for outdoor testing, the performance of these instruments was examined at simulated altitudes (O_2 pressure of <10 kPa) and at temperatures below $-20°C$, while screening the instruments as much as possible from the cold. Most of the different instruments showed systematically too high or too low values (10–25% deviation). The observed differences between instruments may affect the "blood lactate threshold" by 2–5%. We found different readings between "equal" YSI 1500 instruments, while we could see no difference when comparing the other instruments of the same type. Lactate Pro gave reliable results at both $-21 \pm 1°C$ and at simulated altitude. Accusport gave reliable results in the cold, but 1.85 ± 0.08 mmol L^{-1} (mean ± SD) too high readings at the simulated altitude. Of the three simpler instruments examined, the Lactate Pro was at least as good as the YSI instruments and superior to the other two.

Key words: Bicycling; blood; exercise; lactate; lactate threshold; plasma; testing; training

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INTRODUCTION

Blood lactate concentration is often measured in connection with the training and testing of athletes [1, 2] and also of patients [3]. Traditional methods in the laboratory are time-consuming, but now faster and simpler methods have been developed as a consequence of technological development. Today there are several different instruments that can be used to measure the lactate concentration in a small blood sample within a few minutes or less. We have examined the properties and qualities of different instruments.

All measurements are subject to some random error, even if minimized as much as possible. Systematic errors can also occur. One aim of our study has been to compare the imprecision of different instruments and to look for possible systematic errors. In addition, we have looked for possible differences between instruments of the same type by measuring the same blood sample on two or more similar instruments. Two of the instruments examined, the Lactate Pro™ from Arkray in the KDK Corporation and the Accusport® from Boehringer Mannheim, are battery-driven pocket-sized instruments that might be suitable for outdoor testing too. Thus, the performance of these instruments was examined at simulated altitude and in the cold. We also examined instruments from Yellow Springs Instruments (YSI 23L and YSI 1500) and the LP8+ from Dr. Lange. As our reference we used a standard enzymatic photofluorimetric method.

SUBJECTS, EXPERIMENTS AND METHODS

Subjects

Healthy young to middle-aged trained men and women served as subjects in these experiments. All were told that they were serving as volunteers in our experiments. They were also told that they were free to leave at any stage and that they could do so without giving any reason.

Experiments

Most of the exercises were carried out as bicycling on ergometers. Blood samples were taken during treadmill running, rollerskiing, bicycling outdoors and as canoeing or rowing on ergometers. In some experiments, described in more detail elsewhere [2], blood was drawn from catheters in the femoral artery and vein. Otherwise capillary blood was taken from a finger after the hand had been warmed for at least 30 s in tempered water. This procedure increases the perfusion of the hand and makes it easier to get enough blood for several samples in sequence. Sweat contains lactate, and washing in water will also dissolve and thus remove lactate on the skin that otherwise could contaminate the blood. The skin was punctured using a lancet and the first drop of blood was wiped off. Thereafter, blood was taken as described in detail below.

In some experiments, blood samples were taken in conjunction with intensive exercise to exhaustion; in others as part of testing subjects and finding their "blood lactate threshold" and maximal O_2 uptake. Finally, blood samples were taken in some experiments with strenuous exercise with a significant anaerobic energy release, but where the exercise was stopped before exhaustion. We took around 800 blood or plasma samples and measured them using at least two different methods or instruments. Altogether nearly 3000 single measurements of the lactate concentration in blood or plasma samples were carried out.

METHODS

Enzymatic photofluorometry

A volume of 25 or 50 µl of whole blood or plasma was taken by Acupette capillary tubes (P4518-50, Dade Diagnostics Inc., Puerto Rico, USA; according to the manufacturer the accuracy of the tubes' volume is better than 0.5% and the coefficient of variation better than 1%). The fluid was transferred to a tube containing 500 µl of 0.4 mol L^{-1} perchloric acid (PCA) that lyzes the red blood cells and thus frees lactate inside the cells. These tubes were stored frozen at $-20^{\circ}C$ until later analyses of the lactate concentration by a method according to Passoneau and Lowry [4], which uses the increase of NADH concentration in the LDH reaction (lactate dehydrogenase, EC 1.1.1.27; from beef hearts, L2625 type III

from Sigma, St. Louis, MO, USA). Pyruvate produced was further processed by the glutamate-pyruvate transaminase reaction (l-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2; from swine heart, Boehringer Mannheim GmbH, Mannheim, Germany), thus allowing almost complete processing of all lactate in the sample. The fluorescence was read off in an RF-5000 spectrofluorophotometer (Shimadzu, Kyoto, Japan) at 460 nm (652 THz) after 45 min incubation at 23.5°C.

For each analysis, a new second-order standard curve covering the whole range of fluorescence values was made. The error of regression (scatter around the regression line, the statistical error in reading off from the standard curve) was 0.1 – 0.2 mmol lactate L^{-1} blood (or plasma). A standard solution of 1.00 mol lactate L^{-1} (L-lactat(e) standard 125 440, batch 66762401 from Boehringer Mannheim) was used for making the samples for the standard curve. The total imprecision of the method is 0.1 – 0.3 mmol L^{-1}, depending on the lactate concentration in the measured sample. The volume fraction of water in whole blood was taken as 84% and that of plasma as 94% [5].

Since we found systematic differences between the results given by the tested methods and our reference method, the standard solution from Boehringer Mannheim was calibrated independently by an enzymatic reaction similar to that described above [4]. The increase in the NADH concentration was read off in a Shimadzu MPS-2000 spectrophotometer at 340 nm (882 THz) in quartz cuvettes (type 18-B, Starna, Essex, UK), where the light traversed 10.00 mm. The lactate concentration was taken from the measured increase in the NADH concentration using a coefficient of extinction of NADH of 6270 m^{-1} mol^{-1}. These measurements showed that the lactate concentration of the standard solution did not differ from the reported value, and the imprecision (SE) in this calibration was 1%. That imprecision was included when we examined whether there was a systematic difference between a tested instrument and the reference method. Otherwise the possible error in the reference method is considerably less than the systematic deviations given later and is thus regarded in this study as without significance.

YSI instruments

The lactate concentration in samples of 25 µl was measured on an YSI 23L (Yellow Springs Instruments, Yellow Springs, OH, USA) on non-hemolyzed blood and on plasma samples. Further blood analyses were done on five YSI 1500 instruments on either hemolyzed or unhemolyzed 25 µl blood samples. All YSI instruments were calibrated by 5 mmol lactate L^{-1} solutions (YSI 2327). As an additional control, a standard solution of 15 mmol lactate L^{-1} was used (YSI 2328); on some occasions we also used a 30 mmol L^{-1} solution (YSI 1530). In accordance with the instruments' manuals, we required that readings with these solutions gave 5.0 ± 0.1 mmol L^{-1}, 15.0 ± 0.7 mmol L^{-1} and 30.0 ± 1.5 mmol L^{-1}, respectively, before any analyses could be carried out.

YSI instruments assume that a known and constant amount of blood is used for each measurement, and the instruments are equipped with two types of pipettes; one with a needle (YSI 1501 syringepet) and one using capillary tubes (YSI 1502 pipette). In agreement with the manufacturer's instructions we used the same pipette for both the calibrations and the subsequent measurements. When analyses were done on more than one YSI instrument per day, the same syringes and calibration solutions were used for all instruments, and all instruments were in addition handled by the same operator.

In blood, the lactate is found in both plasma and the red blood cells; and in most cases ≈75% of the lactate in blood is found in the plasma compartment [1, 2]. According to the manuals of the YSI instruments, only lactate in plasma is measured unless the blood samples are hemolyzed.

Dr. Lange miniphotometer LP8+

Blood was taken using 10 µl end-to-end capillaries and placed in a reagent solution hemolyzing the blood; lactate was processed in a reaction producing quinonimin in proportion to the amount of lactate in the sample, and the concentration of quinonimin was read off in an LP8+ apparatus (Dr. Bruno Lange GmbH, Berlin, Germany) at 540 nm (576 THz) after a 3 min reaction time.

Lactate Pro

The lactate concentration has been measured in blood and plasma samples on altogether seven different LT-1710 *Lactate Pro*™ analyzers (Arkray Factory Inc., KDK Corporation, Shiga, Japan). This instrument measures lactate on a tiny drop of 5 µl of fluid. The lactate concentration is read off after 60 s. For our measurements we used strips with production numbers L8D04A (*F-4*) and L8L07B (*F-7*).

Accusport

The blood lactate concentration was measured on two different *Accusport*® portable lactate analyzers (type 1488767, Boehringer Mannheim). A drop of no less than 15 µl of blood is applied to the strip [6], and 60 s later the lactate concentration is read off. In accordance with the manufacturer's instructions we checked the "window" on the underside of each strip after each analysis and examined the coloring; if uneven, the analysis was rejected. We also checked that enough blood was added to each strip: When blood is added, some of it penetrates the surface and thus reaches chemicals that start the reactions processing the lactate. We required that there should be liquid blood on the top of the strip's pad after each analysis. Otherwise the result was rejected. We thus used 25–50 µl of blood for each analysis. Blood was added to the strip by letting it drip from a finger, and, in accordance with the instrument's instructions, we never let the finger touch the strip's pad.

This instrument only measures the lactate in plasma; according to the manufacturer, built-in equations calculate the concentration in whole blood from the measured value in plasma [7]. For our measurements we used strips from Boehringer Mannheim with the code number 512.

In concurrence with the instructions for each instrument, all measurements for all instruments were carried out by experienced test leaders who had each done at least a thousand analyses of the blood lactate concentration.

Measurements at simulated altitude

Control solutions from Boehringer Mannheim (article no. 1447335) with a reported lactate concentration of 3.6 mmol L^{-1} (low; BM-control-Lactate 1) and 9 mmol L^{-1} (high; BM-control-Lactate 2) were measured in a chamber where the O_2 pressure was less than 10 kPa, corresponding to an altitude of more than 6 km above sea level. In practice, N_2 gas was led into a bag serving as the chamber. The O_2 pressure in the bag was measured using a Metamax 1 O_2 analyzer (Cortex Biophysic GmbH, Leipzig, Germany), and no analysis was done unless the O_2 pressure was less than 10 kPa. Each standard solution was measured at least 10 times with each of the Lactate Pro and Accusport instruments. As a control, the same control solutions were measured at least 10 times in normal laboratory conditions, that is, at a temperature of 22 ± 1°C and an air pressure of ≈100 kPa, corresponding to an O_2 pressure of ≈21 kPa.

Measurements in the cold

Each of the control solutions from Boehringer Mannheim (3.6 and 9 mmol L^{-1} nominal lactate concentration) was measured at least 10 times on the Lactate Pro and Accusport instruments in a freezing storage room, that is, at a temperature of -21 ± 1°C. The test leader wore a thick sweater and coat. To shield the instruments as much as possible from the cold, the test leader wore a bag under the sweater and thus close to the body. While the strip was inserted into the instrument and the control solution was applied to the strip, actions that took 20–30 s, the instrument and the strip were exposed to the cold. As soon as the analysis started, the instrument was placed in the bag and was thus no longer exposed to the cold. The instrument and the strips lay in the bag between each analysis too. The temperature in the bag was measured with a digital thermometer. It appeared that repeated opening and closing of the bag lowered the temperature, and if it dropped to +5°C or lower the experiments were stopped.

Statistics

The values are given as means ± SD unless otherwise stated explicitly. Different instruments or methods were compared by linear regression using the approach geometric mean that gives equal weight to errors in both series

of measurements [8, 9]; standard linear regression assumes that all error or imprecision is caused by measurements of the ordinate (Y). For the regression analyses carried out here the approach geometric mean gives the same results as a more demanding, robust, and distribution-free approach [10]; the method of Bland and Altman [11] also gives corresponding results for our data. If two instruments or methods differ systematically, the slope will differ from 1.00, and the data points will not be randomly distributed around the line of identity. Thus, possible differences were tested by examining the residual and by standard *t*-tests on the slope. As stated above, we also kept in mind the fact that our reference method may have had a systematic error of ≈1% (SEM). The error of regression (scatter around the regression line) is used as a measure of the random errors. Different errors of regression were tested by Fisher tests [12].

Errors can occur in all measurements. When measurements with two methods or instruments turn out as expected, the values will fall close to a line. If there is something wrong with one of the measurements, the corresponding point will deviate from the linear relationship. A simple scatterplot alone cannot be used to decide whether the deviation seen is due to an error in the abscissa (X) or in the ordinate (Y). For a number of the samples analyzed here each sample was measured using at least three different instruments or methods, here called X, Y and Z. If a point appears as an outlier in the XY plot and the YZ plot but not in the XZ plot, it is likely that the deviation is caused by an error in the Y measurement. We used this principle to unravel possible errors of measurements and outliers.

RESULTS

Blood samples were taken during different experiments, and the blood lactate concentration was measured using at least one of the instruments to be tested (Y) and in addition by enzymatic photofluorometry (X) serving as our reference method (Fig. 1).

Non-hemolyzed blood samples measured on the YSI 23L showed on average a 22% lower value than the reference method ($p < 0.001$; Fig. 1A). Since the YSI 23L does not hemolyze the blood, it does not record lactate in the red blood cells, and this may have caused the systematic deviation. Hemolyzed blood samples were measured on two different YSI 1500, here referred to as the YSI 1500_1 and the YSI 1500_2. The values from the YSI 1500_1 were on average 20% higher than those of the reference method ($p < 0.001$). For the YSI 1500_2 the values were on average 5% higher than those of the reference method; these latter values did not deviate significantly from the line of identity (Fig. 1B, C).

Blood samples were also measured on three different simpler instruments. The values from the LP8+ were on average 26% higher than those of the reference method ($p < 0.001$; Fig. 1D). These measurements also showed a larger error of regression than the others ($p < 0.05$). The relationship to the reference method was convex, and a second-order curve fit was better than a linear one ($p < 0.001$). Values from measurements on the Lactate Pro were on average 12% higher than those from the reference method ($p < 0.001$; Fig. 1E). For all of the instruments mentioned so far, all the regression lines found lie close to the origo. This means that possible systematic errors in the measurements were small for low blood lactate concentrations and rose roughly proportional to the lactate concentration. The Accusport showed two kinds of deviations. First the Y-intercept was close to 1.0 mmol L^{-1} ($p < 0.001$ versus an intercept of 0.0). In line with this, the instrument reported blood lactate concentration around 2 mmol L^{-1} even for blood samples taken at rest, that is, in samples with a true concentration less than 1 mmol L^{-1}. The slope found for this instrument was 0.81, which is considerably less than 1.00 ($p < 0.001$). Thus, this instrument showed correct values for blood samples with a lactate concentration of ≈5 mmol L^{-1}. For samples with less lactate the reported values were systematically too high, while for samples with higher concentration the reported values were too low.

When the so-called "blood lactate threshold" is sought in sports testing, particularly interesting is the performance of the instruments at low to moderate blood lactate concentrations. The same is true for most clinical testing. Therefore blood samples with a lactate concentration above 6 mmol L^{-1}, as measured by enzymatic photofluorometry, were left out and the remain-

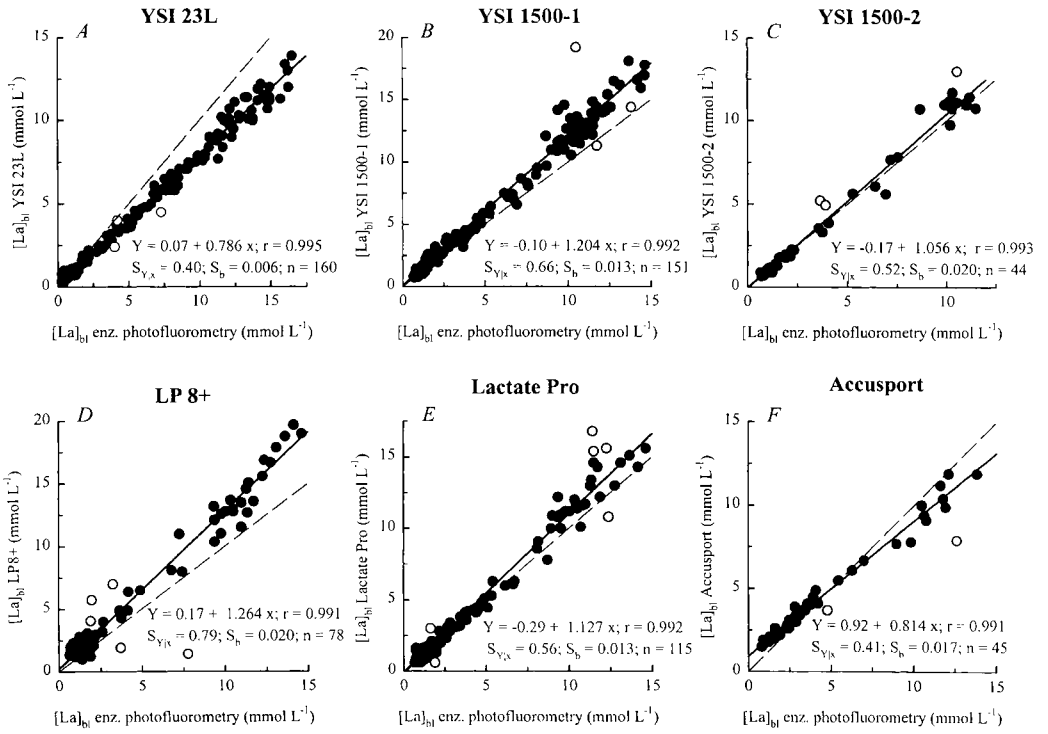
YSI 23L
A
Y = 0.07 + 0.786 x; r = 0.995
S$_{Y|x}$ = 0.40; S$_b$ = 0.006; n = 160
[La]$_{bl}$ YSI 23L (mmol L^{-1})
YSI 1500-1
B
Y = -0.10 + 1.204 x; r = 0.992
S$_{Y|x}$ = 0.66; S$_b$ = 0.013; n = 151
[La]$_{bl}$ YSI 1500-1 (mmol L^{-1})
YSI 1500-2
C
Y = -0.17 + 1.056 x; r = 0.993
S$_{Y|x}$ = 0.52; S$_b$ = 0.020; n = 44
[La]$_{bl}$ YSI 1500-2 (mmol L^{-1})
LP 8+
D
Y = 0.17 + 1.264 x; r = 0.991
S$_{Y|x}$ = 0.79; S$_b$ = 0.020; n = 78
[La]$_{bl}$ LP8+ (mmol L^{-1})
Lactate Pro
E
Y = -0.29 + 1.127 x; r = 0.992
S$_{Y|x}$ = 0.56; S$_b$ = 0.013; n = 115
[La]$_{bl}$ Lactate Pro (mmol L^{-1})
Accusport
F
Y = 0.92 + 0.814 x; r = 0.991
S$_{Y|x}$ = 0.41; S$_b$ = 0.017; n = 45
[La]$_{bl}$ Accusport (mmol L^{-1})
[La]$_{bl}$ enz. photofluorometry (mmol L^{-1})

FIG. 1. The blood lactate concentration measured by enzymatic photofluorometry (*X*) and by six different instruments (*Y*). The samples measured by the YSI 23L (panel *A*) were on non-hemolyzed blood, and these measurements do not record lactate inside the red blood cells. YSI 1500_1 and YSI 1500_2 (panels *B* and *C*) refer to measurements on two different YSI 1500 instruments. LP 8+ (panel *D*) is a lactate analyzer from Dr. Lange, Lactate Pro (panel *E*) is from Arkray in the KDK Corporation, while Accusport (panel *F*) is from Boehringer Mannheim. Data on the regressions are given for each set of data, and the regression lines are shown as solid lines. $S_{Y|X}$ is the error of regression and is expressed in mmol L^{-1}. S_b is the error of the slope. The thin dashed lines show the lines of identity for each set of data. • refers to accepted measurements, while ○ refers to outliers that were formally rejected because of an error of measurement with the instrument examined; these values were therefore not used to calculate the regression lines.

ing data from Fig. 1 were reanalyzed (Fig. 2). For the YSI 23L the relationship for this reduced data set is largely the same as for the full set (Fig. 2A). Thus, also within this range of values the YSI 23L showed values that were too low. The error of regression was roughly half that found for the full data set. Hemolyzed blood samples measured on the YSI 1500_1 showed average values 13% too high ($p<0.001$), while for YSI 1500_2 there was no sign of a systematic deviation (Fig. 2B, C).

The values from LP8+ were on average 25% too high ($p<0.001$; Fig. 2D). The error of regression found for this instrument was about twice that found for the other instruments ($p<0.001$). The Lactate Pro showed no systematic deviations from the reference method for samples with a blood lactate concentration less than 6 mmol L^{-1} (Fig. 2E). For the instruments mentioned so far, the systematic errors were less in both absolute and relative terms for samples with less than 6 mmol lactate L^{-1} blood than for samples covering a larger span. The values from measurements by the Accusport were systematically higher than those from the reference method ($p<0.001$; Fig. 2F).

The LP8+ showed a larger variability than the other instruments (see Figs 1 and 2). We therefore took three parallel measurements on a number of blood samples with this instrument. The median of each measurement was related to measurements with the reference method (Fig. 3A). This approach did not give considerable better values than single measurements as judged from the errors of regression in Figs 1D and 3A.

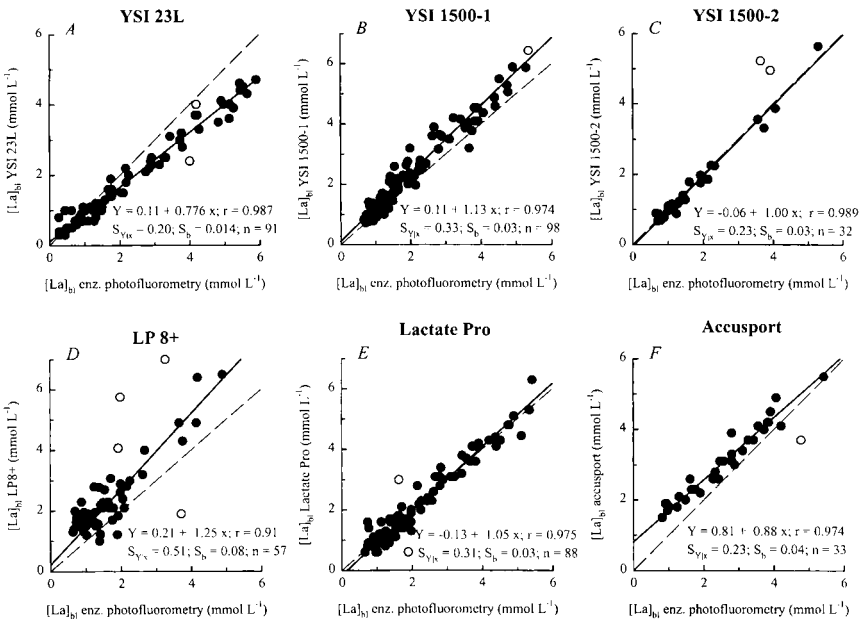
YSI 23L
A
Y = 0.11 + 0.776 x; r = 0.987
S$_{Y|x}$ = 0.20; S$_b$ = 0.014; n = 91
[La]$_b$ YSI 23L (mmol L^{-1})
YSI 1500-1
B
Y = 0.11 + 1.13 x; r = 0.974
S$_{Y|x}$ = 0.33; S$_b$ = 0.03; n = 98
[La]$_b$ YSI 1500-1 (mmol L^{-1})
YSI 1500-2
C
Y = -0.06 + 1.00 x; r = 0.989
S$_{Y|x}$ = 0.23; S$_b$ = 0.03; n = 32
[La]$_b$ YSI 1500-2 (mmol L^{-1})
LP 8+
D
Y = 0.21 + 1.25 x; r = 0.91
S$_{Y|x}$ = 0.51; S$_b$ = 0.08; n = 57
[La]$_b$ LP8+ (mmol L^{-1})
Lactate Pro
E
Y = -0.13 + 1.05 x; r = 0.975
S$_{Y|x}$ = 0.31; S$_b$ = 0.03; n = 88
[La]$_b$ Lactate Pro (mmol L^{-1})
Accusport
F
Y = 0.81 + 0.88 x; r = 0.974
S$_{Y|x}$ = 0.23; S$_b$ = 0.04; n = 33
[La]$_b$ accusport (mmol L^{-1})
[La]$_b$ enz. photofluorometry (mmol L^{-1})

FIG. 2. The blood lactate concentration measured by enzymatic photofluorometry (*X*) and by six different instruments (*Y*). The data are the same as in Fig. 1, but only samples with a blood lactate concentration less than 6 mmol L^{-1} as measured by enzymatic photofluorometry are included. The samples measured by the YSI 23L (panel *A*) were on non-hemolyzed blood, and these measurements do not record lactate inside the red blood cells. YSI 1500_1 and YSI 1500_2 (panels *B* and *C*) refer to measurements on two different YSI 1500 instruments. LP 8+ (panel *D*) is a lactate analyzer from Dr. Lange, Lactate Pro (panel *E*) is from Arkray in the KDK Corporation, while Accusport (panel *F*) is from Boehringer Mannheim. Data on the regressions are given for each set of data, and the regression lines are shown as solid lines. $S_{Y|x}$ is the error of regression and is expressed in mmol L^{-1}. S_b is the error of the slope. The thin dashed lines show the lines of identity for each set of data. ● refers to accepted measurements, while ○ refers to outliers that were formally rejected because of an error of measurement with the instrument examined; these values were therefore not used to calculate the regression lines.

We did further comparisons of measurements on unhemolyzed blood samples measured using the YSI 1500_2 and the Lactate Pro. The values found with the YSI 1500_2 were on average 67% of those found with the Lactate Pro (Fig. 3B). Correcting for a 12% bias in the values from the Lactate Pro (see Fig. 1E) suggests that on non-hemolyzed blood the YSI 1500_2 gives values 25% less than the true value of whole blood. For a further evaluation of the YSI 1500, blood samples were measured using three other instruments, here called YSI 1500_3, YSI 1500_4, and YSI 1500_5, and by the Lactate Pro (Fig. 3C). The values given with these three YSI 1500s did not differ systematically, but for a given value as measured by the Lactate Pro they gave a 13% higher value than the instrument we called YSI 1500_2.

The instruction manual gives no information on how the Lactate Pro reflects lactate in the red blood cells and how it responds to plasma. Therefore plasma from blood samples taken after intense bicycling was measured by enzymatic photofluorometry and by the Lactate Pro. For samples with a lactate concentration less than 10 mmol L^{-1} the Lactate Pro responded as for blood samples, that is, the values found by the Lactate Pro were up to 10% higher than those of the reference method. For samples with a higher plasma lactate concentration the curve leveled off, which means that the Lactate Pro showed too low values (not shown).

Measurements at simulated altitude

The lactate concentration in two control solutions was measured in a chamber with an O_2 pressure less than 10 kPa, corresponding to that found at an altitude of more than 6 km above sea level. The values were compared with

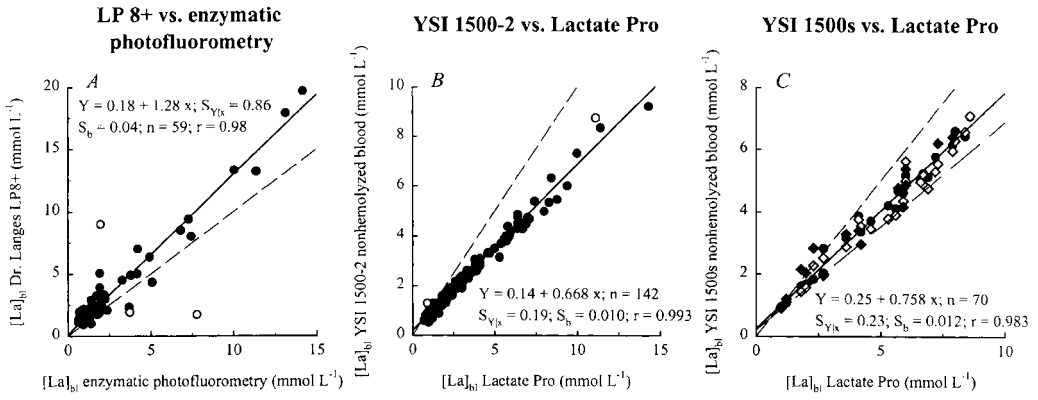
LP 8+ vs. enzymatic photofluorometry
A
Y = 0.18 + 1.28 x; S$_{Y|x}$ = 0.86
S$_b$ = 0.04; n = 59; r = 0.98
[La]$_b$ Dr. Langes LP8+ (mmol L^{-1})
[La]$_b$ enzymatic photofluorometry (mmol L^{-1})
YSI 1500-2 vs. Lactate Pro
B
Y = 0.14 + 0.668 x; n = 142
S$_{Y|x}$ = 0.19; S$_b$ = 0.010; r = 0.993
[La]$_b$ YSI 1500-2 nonhemolyzed blood (mmol L^{-1})
[La]$_b$ Lactate Pro (mmol L^{-1})
YSI 1500s vs. Lactate Pro
C
Y = 0.25 + 0.758 x; n = 70
S$_{Y|x}$ = 0.23; S$_b$ = 0.012; r = 0.983
[La]$_b$ YSI 1500s nonhemolyzed blood (mmol L^{-1})
[La]$_b$ Lactate Pro (mmol L^{-1})

FIG. 3. *A*. The blood lactate concentration measured on LP8+ versus corresponding measurements using enzymatic photofluorometry. The value given for the LP8+ is the median of three parallel measurements. *B*. The lactate concentration measured on non-hemolyzed blood samples on the YSI 1500_2 apparatus versus measurements with the Lactate Pro. ● shows accepted measurements. ○ shows measurements that were rejected because of an incorrect measurement with the instrument examined and therefore not used in calculating the regression parameters. *C*, the lactate concentration measured on non-hemolyzed blood samples on the YSI 1500_3 (●), YSI 1500_4 (◇) and YSI 1500_5 (♦) instruments versus measurements using the Lactate Pro. Regression data are given for each set of data, and the regression lines are shown as solid lines. $S_{Y|x}$ is the error of regression and is expressed in mmol lactate L^{-1} blood. S_b is the error of the slope. The thin dashed lines are the lines of identity. The thin long-dashed line in *C* is a copy of the regression line in *B*.

corresponding measurements at normal O_2 pressure and temperature (Fig. 4). The Lactate Pro showed no effect of reduced O_2 pressure. The values found with the Accusport at reduced O_2 pressure were 1.85 ± 0.08 mmol L^{-1} (mean $\pm$ SD) higher than those found in the control experiments ($p < 0.001$).

Measurements in the cold

The lactate concentration of the control solutions was measured at $-21 \pm 1°C$ (Fig. 4). The instruments were exposed to the cold while a strip was inserted and the control solution was applied to the strips. Otherwise the instruments were well shielded from the cold. The mean values did not differ from those of the control experiments ($p > 0.5$ for each instrument), but the random variations were larger ($p < 0.01$).

DISCUSSION

The main results in this study are first that for all the instruments examined the reported blood lactate concentration rose roughly linearly by the value given by the reference method. Most of the instruments showed either systematically too high or too low values, and the deviations were mainly in the range 10–25%. Apparently equal YSI 1500 instruments showed different values on the same blood samples. Lactate Pro gave reliable results at simulated altitude and in the cold experiments. Accusport gave too high readings at simulated altitude, while the values in the cold were reliable. The LP8+ was the most biased instrument and in addition showed the largest random variations.

Evaluation of the enzymatic photofluorometry method used here

We measured the lactate concentration in blood and plasma by a method that has been in use for more than 30 years in numerous studies. It is common knowledge that the fluorescence from a sample with a known lactate concentration varies from day to day due to interassay variations, and consequently a new standard curve is made for each day of analysis.

While standard curves are usually fitted by linear regression, we used second-order regression. First, a slight curved relationship was readily visible; samples with a high lactate concentration showed a smaller fluorescence than a linear extrapolation dictates. Second, NADH is produced in proportion to the amount of lactate in the sample. There may

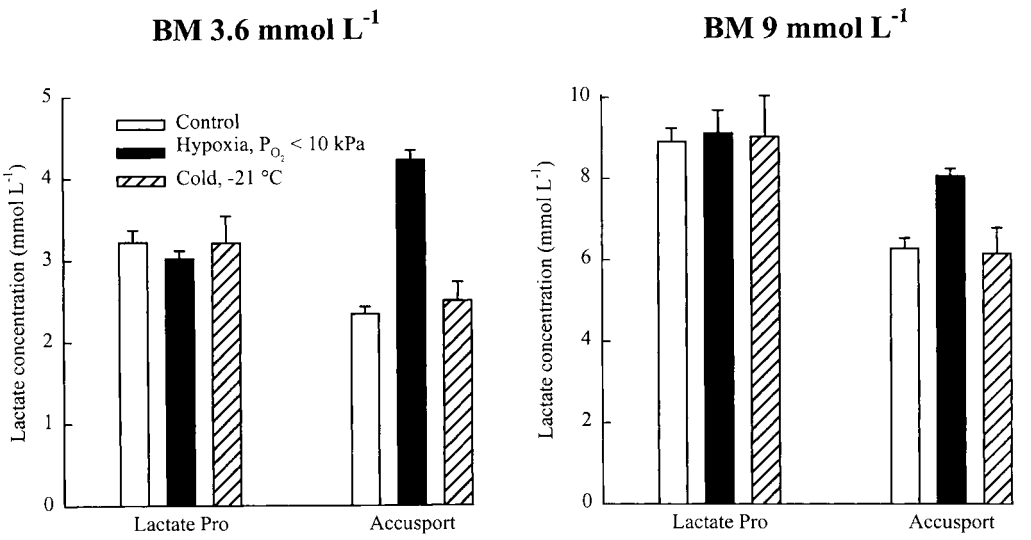

FIG. 4. The lactate concentration of two control solutions from Boehringer Mannheim with a nominal lactate concentration of 3.6 and 9 mmol L^{-1} as measured by the Lactate Pro and the Accusport in control experiments at room temperature and normal air and O_2 pressures at room temperature but with an O_2 pressure below 10 kPa and at normal air and O_2 pressures but at $-21 \pm 1°C$.

be a ≈5% quenching of the signal in a cuvette with a NADH concentration of 10 μmol L^{-1} [4], and since the effect is proportional to the concentration a parabolic relationship is expected. Finally, in samples with a high lactate concentration, 1–2% of the lactate in the sample may not be processed [4]. This latter effect, although quantitatively less important, will add further curvature to the standard curve. There is no reason to assume that our use of second-order curve fits has caused the possible non-linear trends in Fig. 1, since the second-order component of our standard curves was small and in addition tended to reduce rather than increase non-linearities.

We used a commercial 1.00 mol lactate L^{-1} stock solution to establish the standard curve, and separate laboratory controls showed that the concentration of this stock solution was accurate within 2% (95% confidence interval) of the control analyses. A possible bias of less than 2% or random variation less than 0.3 mmol L^{-1} of our reference method has no influence on the conclusions drawn in this study.

Do differences between instruments matter?

When the same sample was measured on two different instruments or by two different methods, we found differences of between 10% and 25%. To examine how this may have affected the so-called blood lactate threshold, that entity, here taken as the exercise intensity giving a blood lactate concentration of 3.00 mmol L^{-1}, was established for one subject during bicycling at 1.5 Hz and found to be 3.42 W kg^{-1} (Fig. 5). To each measured lactate concentration we added (and subtracted) 10% and 25%, which caused the 3.00 mmol L^{-1} "threshold" to appear at 2% and 5% lower (higher) powers, respectively. Systematic differences of that magnitude may be important when judging possible changes for an athlete. Thus, for precise testing of possible changes by time, instruments known to have similar properties should be used.

There are other definitions of the lactate threshold. For blood lactate concentrations higher than 3 mmol L^{-1} the curve is steeper, and if for example the "4 mmol L^{-1} threshold" is used, systematic differences between instruments have less effect. For lower lactate concentration the curve is less steep, and small variations in the measured value will have a larger effect on the reported threshold. For long-distance running, bicycling and skiing the intensity corresponding to a blood lactate concentration of 2 mmol L^{-1} may be of inter-

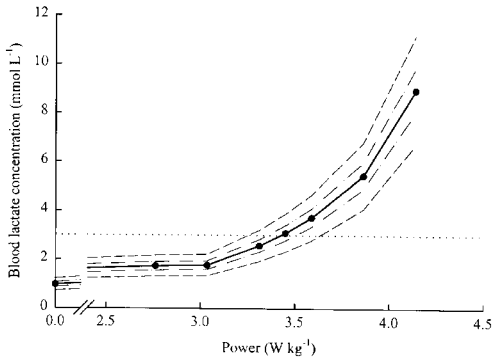

FIG. 5. Measurements of the blood lactate concentration by stepwise increments of the power during bicycling (● and thick solid line). Each step of constant power lasted 5 min, and a 1 min rest separated each step to allow blood sampling and setting a higher power. To each measured value, first 10% (thin dashed-dotted lines) and 25% was added and subtracted (thin dashed lines). The dotted line shows the level of 3.00 mmol L^{-1}, and the power corresponding to that value is here taken as the "lactate threshold".

est. We found that the Accusport showed this value even on samples taken at rest. This instrument should therefore perhaps not be used for that kind of testing. It has also been proposed that an intensity giving a lactate concentration of rest value plus 1.5 mmol L^{-1} should be sought [13]. That definition could be less sensitive to variations between instruments.

Effect of simulated altitude

Control solutions with given lactate concentrations were measured on the Lactate Pro and the Accusport when the O_2 pressure was less than 10 kPa, corresponding to that found at altitudes above 6 km. Readings with the Lactate Pro were not affected by the reduced O_2 pressure, suggesting that this instrument may be well suited for testing at altitude. The Accusport reported too high values in our measurements. Our measurements do not allow us to conclude how this instrument will behave at the O_2 pressures of altitudes of 1500–3000 m that are more typical of athletes in training.

Measurements in the cold

According to the manufacturers, the Lactate Pro and the Accusport may both be unreliable for testing at temperatures below +10°C. Our data suggest that if the instruments and the strips are shielded from the cold, except for the 20–30 s when the strips are inserted in the instrument and blood or test solutions are applied to the strips, the reported values are not affected by surrounding temperatures down to −20°C. Thus, both of these pocket-sized instruments may be just as well suited to outdoor testing as for use in the laboratory. Admittedly, the variations in the measurements being larger in the cold was probably caused by technical problems, since it was more difficult to add a drop of the test solutions from the nipple of the flasks than to add a drop of blood from a fingertip to the strips, particularly for the Lactate Pro in the cold.

Evaluation of the different instruments

Common comments. The error of regression is a simple and usually the best measure of how well the model fits the data. In Fig. 1 the random variations rise by the lactate concentration, and the reported error of regression in that figure is a mean of the error at low and high concentrations. In Fig. 2, where only samples with a concentration less than 6 mmol L^{-1} are included, the error of regression is less, and within the range of data in that figure the random variations seem independent of the concentration.

With the exception of the data on the YSI 23L (Figs 1A and 2A), parallel measurements "on the same blood sample" were done by taking two or more samples from the same finger in sequence, that is, 10–30 s apart, and in random order. The reported error of regression depends on the imprecision in the measurements by enzymatic photofluorometry (X), in the instrument examined (Y), and in possible variations between blood samples taken some seconds apart. The error of regression was least for the YSI 23L. It is likely that the slightly larger errors found for the YSI 1500s, the Lactate Pro and the Accusport are a consequence of small variations between blood samples rather than less precise measurements by these instruments. Statistical considerations suggest that the random error of measurements with each of these three instruments is roughly half of the reported errors of regression in Figs 1 and 2.

YSI instruments. We used one YSI 23L and five YSI 1500s. On non-hemolyzed blood samples the YSIs showed values 20–25% less than the reference method. This finding agrees with data of Foxdal *et al.* [14] and Lormes *et al.* [15]. It is clearly stated in the YSI instrument user manual that if unhemolyzed blood is measured lactate in the red blood cells is not recorded. It is well known that, after a few minutes of equilibration, around 25% of the lactate in blood is found in the red blood cells [e.g. 1, 2]. Thus, one must expect too low apparent blood lactate concentrations when unhemolyzed blood samples are measured.

We did parallel measurements on two different YSI 1500 instruments. Both had fresh membranes, were calibrated using the same solutions and by the same pipette and handled by the same test leader. Nevertheless, these instruments showed a 12% systematic difference on the same samples. Three other YSI 1500s were examined and tested versus the Lactate Pro, which showed a systematic but reproducible bias versus the reference method and was therefore regarded suitable as a control when comparing different YSI 1500s. These three YSI 1500s showed a larger lactate concentration in unhemolyzed blood than the YSI 1500_2, judging from the comparisons with the measurements by the Lactate Pro. We have no explanation for why different YSI instruments gave different results in our experiments. As stated in the Methods section, the instruments were operated by experienced test leaders and in accordance with the instruction manual.

Dr. Lange's LP8+. This instrument showed values 25–30% too high, and the error of regression was nearly twice that of the others. Using the median of three parallel measurements did not reduce the random error much. This instrument costs about the same as the Lactate Pro and Accusport and only 10–15% of that of an YSI 1500. Apart from the lower price than for an YSI 1500 we see no advantage with this instrument compared to the others. This instrument performed less well than the others. It was more difficult to use in terms of handgrips (caps repeatedly off and on), to obtain precise measurements (fill the capillaries completely without spilling blood on the outside), and in terms of not mixing samples in larger series.

Lactate Pro. This instrument showed little bias when blood samples with low to moderate lactate concentrations were measured, while it recorded values 12% too high on samples with a blood lactate concentration above 10 mmol L^{-1}. We saw no systematic difference when the same blood sample was analyzed on different instruments of this type. The random variations in measurements with the Lactate Pro were similar to those of the YSI instruments and the Accusport. Lactate Pro gave reliable results at simulated altitude and in the cold. Shimojo *et al.* [16] have also examined this instrument, and although they found no bias at high lactate concentration, their random variation was larger than ours.

There is no information on how this instrument accounts for lactate in the red blood cells. If it measures lactate in plasma only and adds an assumed value for the lactate in the red blood cells, one would expect too high a reading when fluids without red blood cells are measured. Measurements of the lactate concentration in plasma samples and in control solutions (not shown) with Lactate Pro gave reliable results for solutions with a lactate concentration less than 10 mmol L^{-1}. Thus, this instrument must lyze the red blood cells and thus measures the cell lactate. For non-blood fluids with a lactate concentration above 10 mmol L^{-1} the Lactate Pro gave too low values in our measurements. This instrument may therefore not be suitable for use when fluids other than blood are analyzed and the lactate concentration is high.

Accusport. The Accusport gave correct values only for blood samples with a lactate concentration of $\approx$5 mmol L^{-1}. Even for blood samples taken at rest and with a known concentration less than 1 mmol L^{-1} the Accusport reported values around 2 mmol L^{-1}. When it reported a value of 3.0 mmol L^{-1}, the true value was $\approx$2.5 mmol L^{-1} in our experiments, and that error may be important when testing athletes. Our data suggest that there may be an inbuilt error in this instrument. On examining the Accusport versus a

reference method, Roßkopf *et al.* [17] did not find the bias that we registered, but their random errors were considerably larger than ours.

Two different Accusport instruments gave similar results when the same blood samples were measured. The instrument did not work adequately at simulated altitudes above 6 km, but it did work well in our cold experiments.

The Accusport was more difficult to use than the Lactate Pro, and we got a number of incorrect measurements with it. Some errors could be excluded on grounds of uneven coloring seen through the "window" on the underside of the strip or too little blood applied, but we also experienced errors that could not be detected without comparisons with other analyses. Several measurements were lost because we accidentally hit the on/off-button when the analysis was started by closing the cover. We had technical problems with both instruments. One reported "low battery" and stopped working even when new batteries with the proper voltage were used. The other one could not read the code on the strips used, even when the instrument's window was properly cleaned. Switching batteries and strips between the two instruments showed that the problems lay in the instruments and not in the strips or batteries.

SUMMARY AND CONCLUSIONS

Different instruments gave different values when the lactate concentration of a blood sample was measured. The differences were usually in the range 10–25%, and a bias of this amount may have some effect when athletes are tested. Of the four instruments examined the Lactate Pro was best. The Lactate Pro cost only ≈15% of an YSI 1500 and is much easier to use. Different YSI instruments gave different values. The LP8+ was inferior to the others. Both Lactate Pro and Accusport can be used for outdoor testing in the cold. Lactate Pro can also be used for testing at altitude.

ACKNOWLEDGMENT

We are grateful to Jorid Thrane Stuenæs for her skilled technical assistance.

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Received: 29 February 2000
Accepted: 10 May 2000